

Comments on Possible Consequences of Gene Flow from *Bt* Brinjal to Brinjal Wild Relatives in India, and the Inadequacy of the Current Risk Assessment

- Doug Gurian-Sherman, Ph.D, April 15, 2009^{1,2}

Summary

One important way that genetically engineered (GE) crops may cause environmental harm is through gene flow, which typically occurs when pollen from the GE crop fertilizes either non-GE crop plants, or wild relatives of the crop. In this evaluation, I discuss potential risks to the environment caused by gene flow from *Bt* brinjal to brinjal wild relatives. I also evaluate how Mahyco has addressed this risk, and make some suggestions about how to better determine the gene flow risks from *Bt* brinjal. The conclusions and recommendations in my evaluation are based on existing data on gene flow in the scientific literature, as well as the regulatory and legal record concerning gene flow from GE crops in the United States.

Mahyco presents no data that I am aware of that assesses the risks of gene flow from *Bt* brinjal to wild relatives. The company presents limited data on gene flow distances, which cannot substitute for a risk assessment of potential harm from gene flow, and is wholly inadequate to predict gene flow if *Bt* brinjal were to be commercialized or grown in field trials in areas where wild brinjal relatives are found.

Several wild relatives of brinjal are found in India and have been shown to be sexually compatible with brinjal. And it appears that at least one wild relative grows in or near brinjal fields. Further, methods to prevent gene flow from crops to wild relatives currently do not exist (there are some methods that can slow this process in some crops). Gene flow from *Bt* brinjal to wild relatives, if commercialized, would therefore be virtually certain. Whether the *Bt* gene becomes a permanent part of the environment in India would then depend on the properties of the gene in the wild plants—something that cannot be predicted without performing tests. No such tests have been performed according to the records available to me.³

Harm from gene flow may occur in several ways, but not all gene flow is harmful. If permanent gene flow were to occur, possible harm to the environment can only be imperfectly predicted, and this can only be accomplished by conducting additional tests. These or similar tests have also apparently not been performed.

Because of the high possibility of gene flow from *Bt* brinjal to wild related species, and because India is a center for domestication and genetic diversity of brinjal, I believe that environmental risks due to gene flow from *Bt* brinjal should be seriously considered and evaluated. The only reasonable way to do this is through an understanding

¹ The author has written this evaluation under his own auspices, rather than as a part of any organization.

² I would like to acknowledge invaluable background information and publications from Dr. Marie-Christine Daunay, INRA, France. I also thank Dr. J. Karihaloo for his kind responses to my inquiries about brinjal wild relatives. It must be noted however, that the willingness of Drs. Daunay and Karihaloo to supply information does not constitute an endorsement of the views expressed in this paper.

³ The records that I have examined include: “Field evaluation of *Bt* brinjal in limited field trials during growing season 2002-03,” apparently submitted by Mahyco to GEAC; “Development of Fruit and Shoot Borer Tolerant Brinjal” submitted to the GEAC by Mahyco in 2006. I have also briefly examined other documents submitted to GEAC by Mahyco on toxicology and effects on non-target organisms.

of the biology of wild relatives and their interactions with the environment, and scientific studies including safety tests. Some examples of such studies are outlined at the end of these comments. Because, apparently, none of these studies have been performed, I conclude that the risk assessment of *Bt* brinjal is seriously incomplete, and conclusions about environmental safety are not adequately supported.

Evidence that gene flow from genetically engineered crops may be harmful

The possibility of harm from gene flow has been widely recognized by many scientists.⁴ In the United States, this recognition has been a major factor in regulatory action restricting the commercialization of GE crops with wild relatives. For example, concern about gene flow from GE creeping bentgrass (an important turf grass) to several wild relatives growing in the United States resulted in the U.S. Department of Agriculture (USDA) submitting GE creeping bentgrass to its highest regulatory evaluation—an environmental impact statement (EIS)—under the National Environmental Protection Act. Requiring an EIS had not occurred, I believe, for any other GE crop until that time (this evaluation has been ongoing for several years, and has yet to be completed). Recently, a U.S. Federal Court ruled against the USDA on its regulatory handling of GE creeping bentgrass, largely because of the possibility of gene flow. However, these actions may have been too late to prevent gene flow, which occurred prior to the EIS or court order. The transgene escaped from a large field trial and now appears to be established in the environment.⁵

The geographic restriction of *Bt* cotton cultivation in the United States also demonstrates recognition of the importance of gene flow by U.S. regulatory agencies. The U.S. Environmental Protection Agency (USEPA) prohibits the cultivation of commercial *Bt* cotton in several parts of the southern United States, Hawaii and several Caribbean islands because of the presence in those areas of wild cotton relatives and the possibility that gene flow could cause environmental harm.

The scientific community has also weighted in on the issues of gene flow. The U.S. National Academy of Sciences (NAS 2002) severely criticized the USDA for not adequately assessing possible harm from gene flow of virus-resistance transgenes from GE squash to a wild relative,⁶ and in response USDA supported several studies to retroactively determine whether gene flow could harm the environment. The subsequent studies suggest that these genes may not be harmful in this case. It should be noted that the possibility of harm from gene flow to wild squash was acknowledged and evaluated by USDA prior to commercialization, so the concern by the NAS was that the USDA did not do a thorough assessment. It is not enough to merely acknowledge the possibility of gene flow and to argue that a particular transgene will not cause harm. The regulators

⁴ Ellstrand N, 2003, “*Dangerous Liaisons? When Cultivated Plants Mate with Their Wild Relatives*,” The Johns Hopkins University Press, Baltimore, MD; Snow A.A. et al., 1997, Commercialization of transgenic crops: potential ecological risks, *BioScience* vol. 47:86-96; National Research Council, 2004, “*Biological Confinement of Genetically Engineered Organisms*,” National Academies Press, Washington, D.C.

⁵ Reichman, J.R. et al., 2006, Establishment of transgenic herbicide-resistant creeping bentgrass (*Agrostis stolonifera* L.) in nonagronomic habitats, *Molecular Ecology* vol. 15: 4243-4255

⁶ National Research Council, 2002, “*Environmental Effects of Transgenic Plants: the Scope and Adequacy of Regulation*,” National Academies Press, Washington, DC

must also have adequate data to demonstrate that gene flow either will not occur, or not be harmful if it does.

More recently, the prevention of gene flow to a wild relative of sugar beets was an important part of the regulatory assessment of GE sugar beets by USDA. There is a wild weedy relative of sugar beets in California, and therefore seed production (where flowering is involved and therefore potential gene flow) of GE sugar beets is not allowed in California.

These and other examples, and the scientific literature, establish that gene flow is considered to be one of the most important environmental issues concerning GE crops.

It should also be noted that for the examples discussed above, the United States is not a center of domestication or genetic diversity for any of the crops. However, India is a center of domestication and diversity for brinjal, and this adds additional concern.⁷ This is because centers of domestication usually have particularly high genetic diversity of the crop and wild relatives (which can serve as important sources of genes used in conventional breeding of many vitally important traits, from pest resistance to drought tolerance). This diversity may be harmed by gene flow.

It should also be noted that gene flow to a wild relative will not necessarily cause harm (unless the mere permanent presence of the transgene in the environment is considered to be harmful). However, the only way to determine whether gene flow may cause harm is to perform appropriate tests.

What has been done to determine whether gene flow to wild brinjal relatives may occur, and whether this may be harmful?

Given the widespread concern about gene flow, it is remarkable that there appears to be no assessment of possible harm from gene flow from *Bt* brinjal to wild brinjal relatives in India. The safety record available to me, apparently submitted to GEAC, includes no gene flow safety data.

A few experiments were performed to examine gene flow distances. But this is wholly inadequate, because it is now clearly understood that after commercialization (and possibly also during field trials) gene flow *will* occur if there are wild relatives in the vicinity of the GE crop (whether this becomes permanent depends on the interactions of the transgene with the wild relative and the environment). As noted by the U.S. National Academy of Sciences, currently available technology cannot prevent gene flow from occurring if wild relatives are present.⁸ And since the wild relative *S. insanum*, and possibly *S. incanum*, is found in or near brinjal fields in India, gene flow is a virtual certainty.

It is known that sexually compatible wild relatives of brinjal occur in India. For example, "India: Country Report to the FAO International Technical Conference on Plant Genetic Resources," Leipzig, 1996, notes the presence (page 9) of the sexually compatible wild relatives *S. insanum* and *S. incanum* in India. Dr. J. Karihaloo, an international brinjal expert with the CGIAR, confirmed that *S. insanum* is distributed throughout India, can be found growing in or near brinjal fields as an occasional weed,

⁷ see, for example, "India: Country Report to the FAO International Technical Conference on Plant Genetic Resources," Leipzig 1996

⁸ National Research Council, 2004, op. cit.

and can cross with brinjal to form fertile hybrids, while *S. incanum* may be restricted to Southern Indian scrub forests.⁹ Therefore, *Bt* gene flow is likely to *S. insanum*, and may also occur if brinjal fields are located near scrub forests where *S. incanum* is found. Several other species may also be recipients of gene flow, although this is less clear. Several published papers also establish the sexual compatibility of wild species, and in fact Mahyco itself confirms this.¹⁰

The only data on gene flow appears to be some studies that suggest gene flow occurs up to 15 meters.¹¹ However, gene flow distances are highly dependant on a number of factors such as the size of the experimental plot, presence of pollinators, weather conditions, etc., and tend to be highly variable. Limited tests of the kind done by Mahyco are therefore highly unreliable for accurately predicting gene flow distances.¹² And given that gene flow for brinjal may occur through pollinating insects, and many of these are known to pollinate flowers over a distance of several kilometers, the limited data for brinjal gene flow from Mahyco probably greatly underestimate gene flow distances. These data are wholly inadequate to assess gene flow from multiple large field test plots or commercialized *Bt* brinjal.

A clear example of the failure of these kinds of gene flow distance predictions was the proposed isolation distance to separate GE creeping bentgrass from wild relatives in the United States. This isolation distance was initially set at 900 feet. Subsequent studies by USEPA scientists indicated that gene flow actually occurred at distances of at least 21 km from a large field trial.¹³ This and many other examples show that limited experiments to determine adequate isolation distances (as done by Mahyco) should be viewed with a very high degree of skepticism.¹⁴

If the *Bt* gene makes a wild crop relative more able to survive and reproduce than the wild relative without the transgene (in genetics terms, makes the wild relative more fit), then population genetic theory clearly shows that the gene will probably spread through the wild-relative population and become common in it and a permanent part of the environment.¹⁵ When this occurs, it becomes virtually impossible to eradicate the gene from the natural environment, and therefore, if it is harmful, that harm is highly likely to become a permanent feature of the environment where the wild plants grow.

Once gene flow has occurred and the gene has become established in the wild relative population, harm can occur in several ways, depending on the properties of the gene, the recipient wild relative, and the environment. If the wild relative containing the transgene is more competitive toward other plant species than without the gene, it will develop larger populations and to some extent replace other plants in the environment. If the wild relative is an agricultural weed, then it may become a more serious weed causing

⁹ J. Karihaloo, personal email communication with D. Gurian-Sherman, February 11, 2007

¹⁰ see "Development of Fruit and Shoot Borer Tolerant Brinjal" submitted to the GEAC by Mahyco in 2006

¹¹ "Development of Fruit and Shoot Borer Tolerant Brinjal," op. cit.

¹² Gurian-Sherman, D., 2006, Contaminating the Wild? *Gene Flow from Experimental Field Trials of Genetically Engineered Crops to Related Wild Plants*, Center for Food Safety, Washington, DC; Ellstrand N, 2003, "*Dangerous Liaisons?*" op. cit.

¹³ Watrud L et al, 2004, Evidence for landscape-level, pollenmediated gene flow from genetically modified creeping bentgrass with CP4 EPSPS as a marker, *Proceedings of the National Academy of Sciences USA* 101(40):14,533-14,538

¹⁴ Gurian-Sherman, D., 2006, Contaminating the Wild? op. cit.

¹⁵ National Research Council, 2004, op. cit.; Ellstrand N, 2003, "*Dangerous Liaisons?*" op. cit.

greater crop losses. An important wild relative of *Bt* brinjal that can be the recipient of the *Bt* gene is *S. insanum*. This plant is widely dispersed in India and may be found in and near brinjal fields as an occasional weed, and therefore should be evaluated for its weed potential if it received a *Bt* gene.

The *Bt* gene may make wild brinjal relatives become more aggressive weeds by reducing damage from insects that are susceptible to the *Bt* toxin, especially moth larvae of various species. Preliminary data suggesting this kind of effect was reported in a peer-reviewed paper showing that a weedy wild relative of sunflowers that contained the *Bt* gene survived and produced more progeny than the normal wild sunflowers without the *Bt* gene.¹⁶ This research suggests that if gene flow occurred from the *Bt* sunflower crop to wild sunflower relatives, the gene would spread through the wild population, and possibly cause environmental harm, such as by making the wild sunflowers more aggressive weeds (*Bt* sunflower has not been approved or commercialized in the United States). Unfortunately, the company that owns the *Bt* gene forbade any further research using their *Bt* gene, so we still do not know how much harm wild *Bt* sunflowers could cause. Whether a similar situation may occur with *Bt* brinjal can only be determined by performing the proper tests.

Another way that gene flow may cause environmental harm is if the toxin kills organisms that use the wild brinjal relatives as a food source. This is most likely to occur with moths and butterflies, but may also occur with other organisms.¹⁷ For example, laboratory studies have shown that *Bt* toxins may harm a number of different insect species.¹⁸ A recent scientific review, for example, pointed out that some species of moths or butterflies are highly dependent on wild sunflowers as a food source, and if they are killed or harmed by the *Bt* gene, those species could face serious problems if the gene spread through the wild sunflower populations.¹⁹ Again, whether this may be the case with wild brinjal relatives in India can only be determined by the appropriate tests.

Gene flow may also reduce the genetic diversity of wild crop relatives, and these wild relatives may be sources of genes that could be important for improving brinjal. There are several known instances of loss of genetic diversity by wild relatives due to large amounts of gene flow from the nearby crop (known examples are non-GE, but could also happen with GE).²⁰ A gene that confers a powerful selective advantage (as *Bt* may) might exacerbate such effects.

What should be done?

It is clear that gene flow (at least the initial stages that lead to hybridization) from brinjal to wild relatives in India is likely to occur, at least to *S. insanum*, and possibly also

¹⁶ Snow AA et al., 2003, A *Bt* transgene reduces herbivory and enhances fecundity in wild sunflowers, *Ecological Applications* vol.13:279-186

¹⁷ It should be noted that while some moths are important agricultural pests, many are important plant pollinators.

¹⁸ Lövei, G.L. and S. Arpaia, 2004, The impact of transgenic plants on natural enemies: a critical review of laboratory studies, *Entomologia Experimentalis et Applicata* vol. 114: 1–14

¹⁹ Pilson D. and Prendeville H.R., 2004, Ecological effects of transgenic crops and the escape of transgenes into wild populations, *Annual Review of Ecology Evolution and Systematics* vol. 35:149-174

²⁰ Ellstrand N, 2003, “*Dangerous Liaisons?*” op. cit.

to *S. incanum*. It is also clear from the literature and scientific consensus that gene flow may cause environmental harm. Therefore, the possibility of the *Bt* gene entering wild brinjal relatives and causing harm should be taken seriously. What should therefore be done to determine if permanent gene flow may occur and whether it would cause harm?

It is beyond my intention to suggest a detailed testing regime that should be carried out to determine whether *Bt* brinjal could cause environmental harm, but a few brief examples may illustrate the kinds of studies that could be done, have been done in the United States, or have been suggested by scientists.

First, better data could be gathered as to the frequency of gene flow to *S. insanum* and *S. incanum*, and the fertility and fitness of hybrids between them and brinjal. The fitness of the gene in the wild relative itself should also be determined. The main focus for *S. insanum* should probably be on fitness determinations, because the data already available suggest that gene flow to this relative is probably inevitable (although more data on gene flow would also be useful). Fitness determinations would give an idea of the likelihood of the gene spreading through wild relative populations (this was done in the U.S., belatedly, for a squash wild relative concerning possible gene flow of virus resistance genes). For *S. incanum*, it appears that more data are needed to determine whether it occurs in areas where brinjal is planted. If so, the fitness of *Bt* brinjal:*S. incanum* hybrids, and *S. incanum* containing the *Bt* gene, should be determined.

Second, if the *Bt* gene increases the fitness of wild relatives, it should be determined if this increases the competitiveness of those relatives compared to other plants that grow with it, and also if *Bt* enhances the weediness of the wild relative.

Third, it should be determined whether wild relatives are important food sources for insects that feed on it, especially moths and butterflies. This was done in the United States after it was found that the *Bt* gene may harm the highly valued monarch butterfly (in that case it was found that the most widely commercialized varieties of *Bt* corn probably are not harmful to monarchs in the wild, but another type that had more toxin in its pollen, could probably have caused harm if it had been more widely grown).²¹ If the wild brinjal relatives are important food sources, those insect species that rely on brinjal should be tested for susceptibility to the *Bt* toxin.

These tests, broadly speaking, are noted mainly to highlight the glaring lack of any tests in the submissions of Mahyco, as far as I can ascertain. It would be, in my opinion, a major oversight on the part of the GEAC to allow the commercialization of *Bt* brinjal, or large-scale field trials, without better understanding the implications of gene flow.

Sincerely,
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²¹ Stanley-Horn D.E. et al., 2001, Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies, *Proceedings of the National Academy of Sciences USA* vol. 98: 11,931–11,936